Journal of Chromatography, 547 (1991) 175–183
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 209

Combination of ion-pair and column switching in high-performance liquid chromatography of tropane alkaloids

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(First received November 5th, 1990; revised manuscript received February 5th, 1991)

ABSTRACT

Ion-pair high-performance liquid chromatography was combined with column switching to determine tropane alkaloids, mainly hyoscyamine, in complex preparations of gastrointestinal drugs using a three-pump system. The mobile phases were all the same except for the concentration of the counter ion, sodium dodecyl sulphate (SDS). The tropane alkaloid fraction immediately eluted by the primary mobile phase without SDS from a pretreatment column was transferred to an analytical column and separated by the ion-pair mobile phase. A tertiary pump was used to supply SDS to the trapped fraction in the loop after elution through the pretreatment column. Linear calibration graphs were obtained in the range 0.3–30 μg/ml for anisodine, anisodamine and scopolamine and 0.3–300 μg/ml for hyoscyamine (atropine). Hyoscyamine levels in model preparations of gastrointestinal drugs were determined by this column switching system with a recovery of about 97%.

INTRODUCTION

Crude drugs derived from solanaceous plants containing tropane alkaloids and their extracts, such as scopolia and belladonna extracts, are used in antidiarrhoeal and gastrointestinal drug preparations as anodynes and anticonvulsants. Scopolia extract is an important ingredient of gastrointestinal drugs and other crude drugs are often prepared with scopolia extract. The determination of tropane alkaloids, mainly hyoscyamine and scopolamine, has been attemped by chromatographic techniques such as thin-layer chromatography [1], gas chromatography [2,3] and high-performance liquid chromatography (HPLC) [4–14]. HPLC is a useful technique for the determination of tropane alkaloids from the viewpoint of resolution and sensitivity. Although several HPLC methods have been applied to solanaceous crude drugs and their extracts and preparations, time-consuming pretreatment, such as partitioning

0021-9673/91/$03.50

between alkaline ammonia and chloroform or diethyl ether, is required, particularly for preparations. This is because the common combination dosage of scopolia extract is low and impurities from large doses of other crude drugs or ingredients interfere chromatographically.

Recently we developed two ion-pair HPLC systems. One is for the determination of four tropane alkaloids [13], anisodamine, anisodine, hyoscyamine and scopolamine, but it requires alkaline ammonia–chloroform extraction. The other is a simple and rapid method [14] with no complicated pretrearments required. The advantage of ion-pair chromatography is that the retention time of an ionic compound can be controlled by changing the type and concentration of the counter ions and the pH of the mobile phase. However, even these ion-pair chromatographic methods are not applicable to preparations containing some crude drugs without complicated pretreatment.

The column switching system [15] can be used for on-line sample clean-up and the analysis of complex preparations and trace amounts of urine or blood analytes, etc. Highly selective separations can be achieved by changing transfer techniques and/or switching functions or by altering the chromatographic modes of separation during the overall process. One simple approach is to transfer selectively a desired fraction eluted from a primary to a secondary column for further separation.

Coupling these two techniques permits the on-line determination of tropane alkaloids in complex preparations with no complicated pretreatment. In this study, application of combined ion-pair and column switching to the determination of tropane alkaloids, mainly hyoscyamine, in complex preparations is described.

EXPERIMENTAL

Materials

Scopolia acutangulus was collected in Yunnan. *Scopolia japonica, Duboisia leichhardtis*, fennel, plectranthi herb, scutellaria root, glycyrrhiza, cinnamon bark, gentian, geranium herb, amomum seed, ginger, swertia herb, rhubarb, ginseng, alpinia rhizome, evodia fruit, corydalis tuber, phellodendron bark, sophora root and magnolia bark were available from markets. Scopolia extract was according to the Pharmacopoeia of Japan.

Chemicals and reagents

Atropine hydrosulphate (as hyoscyamine) and scopolamine hydrobromide standards were purchased from Wako (Osaka, Japan). Anisodine and anisodamine were isolated and purified at the Bejing Institute of Materia Medica. Methanol for chromatography, phosphoric acid, sodium phosphate dihydrate and sodium dodecyl sulphate (SDS) of biological grade were obtained from Wako.

Apparatus

The Shimadzu LC-6AD system consisted of a SIC chromatocoder 12 computing integrator, three pumps (two Shimadzu LC-6ADs for the primary and secondary pumps and a Waters M-45 for the tertiary pump), a Shimadzu SIL-6A system controller, a Rheodyne 7001 pneumatic switching valve and a Shimadzu SPD-6A UV detector. The pretreatment column (50 mm × 4 mm I.D.) and analytical column (250

mm × 4 mm I.D.) were packed with TSK Gel 120A, 5 μm (TOSOH, Tokyo, Japan) by slurry packing. The loop volume was 1 ml.

HPLC conditions

The primary mobile phase for the pretreatment column was a mixture of 1/15 *M* sodium phosphate solution (adjusted to pH 3.5 with 1/15 *M* phosphoric acid) and methanol (48:52). The secondary mobile phase for the analytical column was the primary mobile phase containing 17.5 m*M* SDS. The tertiary mobile phase was the primary mobile phase containing 175 m*M* SDS. The column temperature was maintained at 35°C and the flow-rates of the primary, secondary and tertiary mobile phases were 0.9, 1.0 and 0.1 ml/min, respectively. The eluted substances were detected by a UV detector at 210 nm.

Sample preparation and assay procedure

Solanaceous plants. Dry powders of each crude drug (0.5 g) were refluxed for 30 min in 25 ml of the primary mobile phase, cooled, centrifuged at 1600 *g* and decanted. The residue was washed twice with 10-ml of the primary mobile phase. The extract and washings were diluted to 50 ml with the primary mobile phase. A 20-μl portion of this solution was injected into the pretreatment column of the HPLC system. The eluate fraction from 0.25 to 1.25 min was trapped in the loop and transferred to the analytical column. The hyoscyamine, scopolamine, anisodine and anisodamine concentrations were calculated from the relevant peak areas.

Other crude drugs. Using appropriate amounts of the crude drugs listed in Table I and water–methanol (48:52) as the extraction solvent instead of the primary mobile phase, the sample solutions were prepared as described for the solanaceous plants.

Model preparations. The corresponding amounts listed in Table II of each crude drug sample solution were mixed, evaporated and dissolved in 50 ml of the primary mobile phase to result in model preparations I and II. Each blank model preparation was prepared in the same manner but without scopolia extract. A 20-μl aliquot of each sample solution was analysed with the column switching system and also with the analytical column alone with the secondary mobile phase.

TABLE I

CRUDE INGREDIENTS OF GASTROINTESTINAL DRUG PREPARATIONS

Crude drug	Sample amount (g)	Crude drug	Sample amount (g)
Ginger	1.0	Cinnamon bark	1.0
Glycyrrhiza	1.5	Fennel	1.0
Alpinia rhizome	1.0	Plectranthi herb	0.5
Ginseng	1.5	Scutellaria root	3.0
Amomum seed	0.1	Swertia herb	1.0
Gentian	0.2	Geranium herb	1.5
Corydalis tuber[a]	0.8	Rhubarb	0.2
Evodia fruit[a]	1.0	Phellodendron bark[a]	3.0
Magnolia bark[a]	1.5	Sophora root[a]	1.5

[a] Crude drug contains alkaloids.

TABLE II

PRESCRIPTION OF MODEL GASTROINTESTINAL DRUGS

Model preparation I		Model preparation II	
Component	Amount (g)	Component	Amount (g)
Ginger	0.1	Swertia herb	0.05
Cinnamon bark	0.3	Gentian	0.2
Fennel	0.2	Geranium herb	0.5
Amomum seed	0.1	Phellodendron bark	0.5
Ginseng	0.3	Plectranthi herb	0.5
Glycyrrhiza	0.15	Scopolia extract	0.03
Scopolia extract	0.03		
Recovery of hyoscyamine (%)	96.6		97.0
R.S.D. (%, $n = 5$)	1.2		1.7

Calibration graphs

Calibration graphs for hyoscyamine, scopolamine, anisodine and anisodamine obtained by the column switching system were obtained over the ranges 0.6–322.0, 0.3–31.0, 0.3–30.0 and 0.3–33.0 μg/ml, respectively, and the corresponding regression equations were $y = 17700x - 550$ ($r = 0.999$), $y = 16020x + 570$ ($r = 0.999$), $y = 15280x + 440$ ($r = 0.999$) and $y = 14680x - 680$ ($r = 0.999$).

RESULTS AND DISCUSSION

HPLC conditions

A column switching system using two columns for pretreatment and analysis generally includes the fractional transfer of the effluent from the pretreatment column. As the fraction to be transferred is a front, middle or end eluting zone, a large volume, sometimes up to 1 ml, of the effluent is transferred. Although the chromatographic modes in the column switching system can be controlled by changing the composition of the mobile and/or the stationary phases, when the same stationary phase is used for both the pretreatment and analytical columns, considerably different mobile phases are required. In this instance, the composition of the effluent must be very similar to that of de secondary mobile phase for subsequent analysis, because a large volume of the effluent from the pretreatment column, which has a different solvent system, strongly affects the separation efficiency of the analytical column. Adjusting the composition of the effluent to be transferred by adding water or organic solvent after elution from the pretreatment column causes an increase in volume. The retention of ionic compounds such as tropane alkaloids can be controlled by adding a counter ion to the mobile phase. Therefore, the addition of a small volume of solvent containing a high concentration of counter ions to adjust the final counter ion concentration can minimize this disadvantage. Our separation strategy was as follows: tropane alkaloids were eluted immediately from the ODS pretreatment column by the primary mobile phase without the counter ion, and the separated on the ODS analytical column under ion-pair conditions.

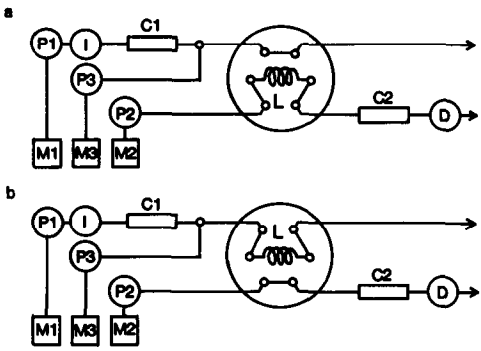

Fig. 1. Schematic diagram of the column switching system. (a) Waiting and analysis mode; (b) trapping mode. P1, P2, P3 = pumps; C1 = pretreatment column; C2 = analytical column; D = detector; L = loop; M1 = primary mobile phase; M2 = secondary mobile phase; M3 = tertiary mobile phase.

There are several transfer techniques for introducing a desired fraction into a secondary column, namely direct, indirect, reversed, loop and backflush. Direct or loop transfer techniques were suitable for this system. Loop transfer was examined to avoid an increase in column pressure when the pretreatment and analytical columns were connected. This system is shown in Fig. 1. The ion-pair chromatographic conditions for the secondary column were similar to those in our previous study [13]. A 5-cm long pretreatment column was used to minimize the transfer fraction volume. Four tropane alkaloids were eluted from 0.4 to 1.1 min (Fig. 2a). A 25-cm long column was used for analysis. The loop volume was 1.0 ml for complete alkaloid transfer. First, 1.0 ml of the eluate from the pretreatment column without SDS was directly transferred to the analytical column, but split peaks of tropane alkaloids appeared, as shown in Fig. 2b. It seems that this phenomena was due to an insufficient counter ion effect. A tertiary pump as used to supply SDS before the analytical column. The tertiary pump solution contained ten times more SDS. After trapping the sample, the final SDS concentration in the loop mobile phase was maintained at 17.5 m*M*, the same as in the secondary mobile phase, by keeping the flow-rate constant at 10% of the total. The flow-rates were set at 0.9, 1.0 and 0.1 ml/min for the primary, secondary and tertiary mobile phases, respectively. The ratio of the buffer to methanol was the same in all mobile phases. As shown in Fig. 2c, a clearly separated peak was obtained. When the eluted fraction from 0.25 to 1.25 min after the injection was transferred to the analytical column, the recoveries of hyoscyamine, scopolamine, anisodine and anisodamine from the pretreatment column were 99.5% [relative standard deviation (R.S.D.) = 0.5%], 97.7% (R.S.D. 0.6%), 100.0% (R.S.D. 0.6%) and 98.8% (R.S.D. 0.5%), respectively, with $n = 5$.

Analysis of solanaceous plants

Tropane alkaloids in *Scopolia japonica, Scopolia acutangulus* and *Duboisia leichhardtis*, which had been analysed by ion-pair HPLC after ammonia–alkaline chloroform extraction, were separated clearly by this column switching system (Fig. 3). The analytical results showed good agreement with our previous data [13].

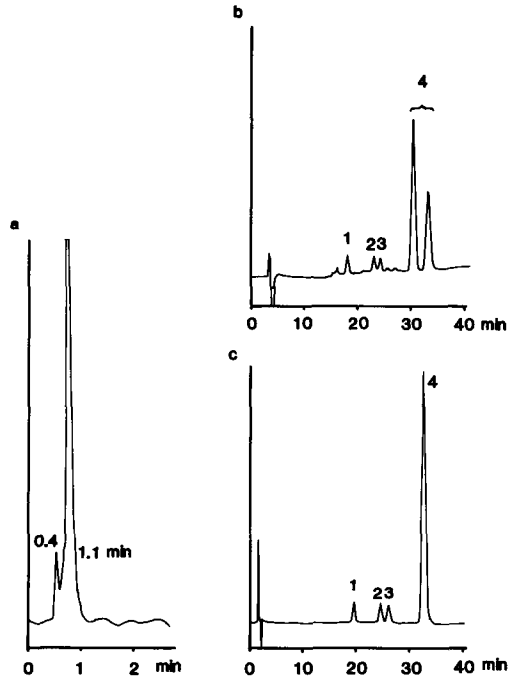

Fig. 2. (a) Chromatogram of the mixture of the four tropane alkaloids from the pretreatment column. (b) Chromatogram of the four tropane alkaloids by column switching without tertiary mobile phase. (c) Chromatogram of the four tropane alkaloids by column switching. Peaks: 1 = anisodine; 2 = anisodamine; 3 = scopolamine; 4 = hyoscyamine. Injection volume, 20 μl. Standard solution contains anisodine, anisodamine and scopolamine at concentrations of 10 μg/ml each and hyoscyamine at 160 μg/ml.

Analysis of model preparations

Scopolia extract is an important ingredient of gastrointestinal drug mixtures, and contains hyoscyamine and scopolamine. Many kinds of crude drugs are often combined with scopolia extract in gastrointestinal preparations. Representative crude

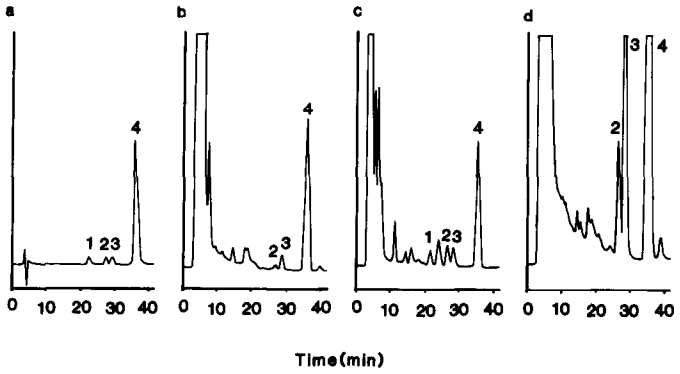

Fig. 3. Chromatograms of solanaceous crude drugs. (a) Standards; (b) *Scopolia japonica*; (c) *Scopolia acutangulus*; (d) *Duboisia leichhardtis*. Peaks as in Fig. 2.

drugs are given in Table I. The dosage form of scopolia extract is lower than that of other crude drugs. The determination of the tropane alkaloids in these gastrointestinal drugs is difficult without complicated pretreatment. Therefore, the present column switching system was applied to the analysis of a complex gastrointestinal drug preparation using two model mixtures. Table II lists the prescription of the model gastrointestinal drugs. The model preparation II has an alkaloid crude drug, phellodendron bark, and the other does not. Corresponding blank model preparations that contained no scopolia extract were prepared to check the interferences at the position of tropane alkaloids on the chromatograms.

Figs. 4 and 5 show the chromatogram of these two model preparations. In the model preparation I and the corresponding blank, there were many interfering peaks from other crude drugs from the analytical column without a clean-up procedure. Therefore, no tropane alkaloids were directly determined (Fig. 4a and a'). However, most interferences from other crude drugs were removed by the column switching procedure in the full model preparation I (Fig. 4b). No peak appeared at the position of hyoscyamine on the chromatogram of the blank model preparation I (Fig. 4b'). It was difficult to determine scopolamine because of the low concentraions. Regarding the analysis of the model preparation II, phellodendron bark seemed to interfere with the hyoscyamine and scopolamine peaks, as berberine-type alkaloids in phellodendron bark would show a similar behaviour to tropane alkaloids and would not be removed by the pretreatment column. However, most berberine-type alkaloids were

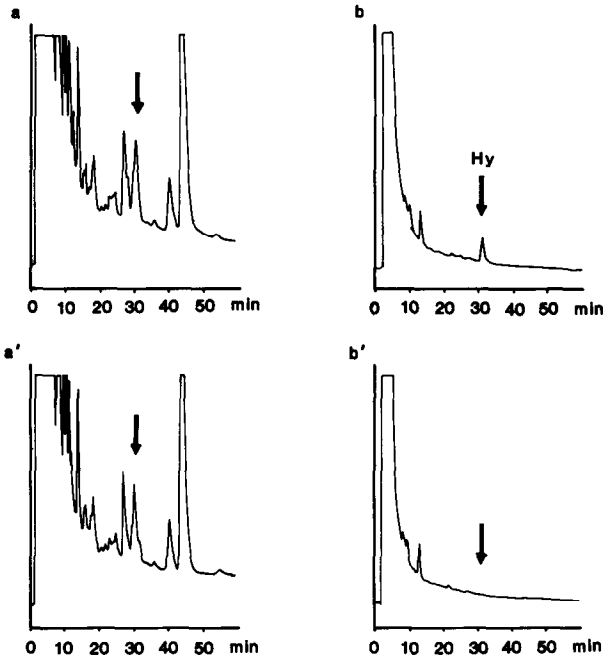

Fig. 4. Chromatograms of (a) model preparation I without clean-up procedure, (a') blank model preparation I without clean-up procedure, (b) model preparation I by column switching, (b') blank model preparation I by column switching. Peak: Hy = hyoscyamine.

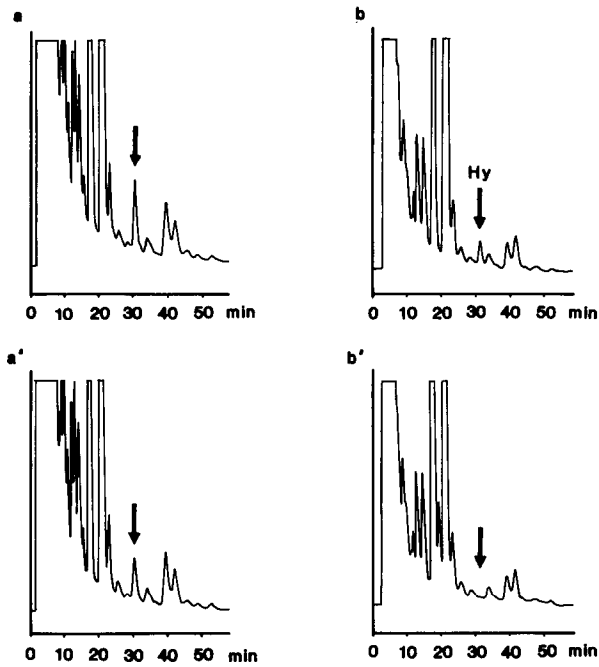

Fig. 5. Chromatograms of (a) model preparation II without clean-up procedure, (a') blank model preparation II without clean-up procedure, (b) model preparation II by column switching; (b') blank model preparation II by column switching. Peak: Hy = hyoscyamine.

separated from hyoscyamine on the analytical column. As shown in Fig. 5a and a', hyoscyamine interfered with the blank model preparation II without clean-up, but this peak was removed by the column switching system. (Fig. 5b and b'). Glycyrrhiza and amomum seed were the interferences in the model preparation I and gentian in the model preparation II. The recoveries of hyoscyamine from scopolia extract in the model preparations I and II were 96.6 and 97.0%, respectively. In addition to the model preparations, several crude drugs that could be combined with scopolia extract in gastrointestinal drug preparations were checked and no significant interference of hyoscyamine was found.

CONCLUSIONS

The determination of tropane alkaloids, especially hyoscyamine, in complex preparations was achieved by combining ion-pair chromatography and column switching using the third pump to supply SDS before transfer to the analytical column. This system seems to be applicable to the on-line determination of other ionic components in complex preparations.

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